EXPRESS MAIL NO. EL 683235780US

JC05 Rec'd PCT/PTO 19 JUL 2000

FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY 'S DOCKET NUMBER: 7250-11

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U S APPLICATION NO (If known, see 37 CFR 1 5): 09/889717

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/00144	January 21, 2000	January 21, 1999

TITLE OF INVENTION: FIBRES

APPLICANT(S) FOR DO/EO/US: Douglas W. Hamilton; Christopher L. Ives; Ian P. Middleton; Chiara Rossetto

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☒ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned)
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☒ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:
    International Preliminary Examination Report

"Express Mail" label number EL 683235780 US
Date of Deposit July 19 2000
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.
Gail Mercer
Signature of person mailing paper or fee

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/889717	INTERNATIONAL APPLICATION NO PCT/GB00/00144	ATTORNEY'S DOCKET NUMBER 7250-11

21. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	28 - 20 =	8	x **$18.00**	$ 144.00	
Independent claims	3 - 3 =	0	x **$80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1004.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$	
SUBTOTAL =				$ 502.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 502.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 502.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 502.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3030 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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09/889717
JC18 Rec'd PCT/PTO 19 JUL 2001

7250-11:135453

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:)
)
Douglas William Hamtilon et al.)
)
Serial No. (unknown))
)
Filed Herewith)
)
FIBRES)
)
US National Stage of PCT/GB00/00144)
International Filing Date January 21, 2000)

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents

Washington, D.C. 20231

Sir:

Please enter the following Preliminary Amendment in the above-identified patent application. The Commissioner is hereby authorized to charge payment of any additional fees associated with this application or credit any overpayment to Deposit Account No. 23-3030.

IN THE CLAIMS

Please amend the claims to read as follows:

"Express Mail" label number EL683235780US. Date of Deposit July 19, 2001
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Trademarks, 2900 Crystal Dr., Arlington, Virginia 22202-3513.

Gail Mercer
Signature of Person Mailing Correspondence

Clean Copy of Amended Claims

1. A method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

2. A method as claimed in claim 1 wherein the fibres have a length of 5mm-500mm.

3. A method as claimed in claim 1 wherein the fibres have a diameter of 5μm-1000μm.

4. A method as claimed in claim 1 wherein the depth of said channels is at least 1μm but not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre.

5. A method as claimed in claim 1 wherein the width of the channel is no greater than half the radius of the fibre.

6. A method as claimed in claim 1 wherein the channel formation extends longitudinally along the fibre.

7. A method as claimed in claim 1 wherein the channel formations extend transversely to the longitudinal axis of the fibre.

8. A method as claimed in claim 1 wherein channel is of U- "square-U", "rectangular-U" or V-shaped cross-section.

9. A method as claimed in claim 6 wherein, in transverse cross-section, the fibres comprise a plurality of lobes and said channel formation is defined between lobes of the fibre.

10. A method as claimed in claim 1 wherein the cells locate on the open-mouths of the channel.

11. A method as claimed in claim 10 wherein said channel provides for guided growth of the cell along the channel.

12. A method as claimed in claim 1 wherein the channels are dimensioned such that cells locate wholly within the channel.

13. A method as claimed in claim 1 wherein the channels are dimensioned such that cells locate partly within the channel and partly above the profile of the fibre.

14. A method as claimed in claim 1 wherein the fibres are in the form of a scaffold.

15. A method as claimed is claim 14 wherein the fibres are orientated.

16. A method as claimed in claim 15 wherein the fibres of different composition are layered.

17. A method as claimed in claim 1 wherein the fibres are aligned as parallel on a permeable flat surface.

18. A method as claimed in claim 1 wherein said cells are selected from chondrocytes, cardiomyocytes, osteoblasts, myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchymal origin.

19. A fibre with an open-topped channel formation the depth of the channel being no more than 2/3 the diameter of the fibre but at least the width of an unspread cell (normally 10-20μ) and width no greater than ½ the radius.

20. A fibre as claimed in claim 19 that allows for cell adhesion and guided migration and growth.

21. A fibre with an open-topped channel formation in the form of a trough where the trough is at least 20 microns wide and 20 microns deep.

22. A fibre as claimed in claim 21 wherein the trough extends along the length of the fibre.

23. A fibre as claimed in claim 19 in which various levels and gradients types of growth factor have been entrapped allowing diffusion to the surface to control growth.

24. A microfibrous cell scaffold composition comprising a fibre as claimed in claim 19 for growing cells to produce functional tissue replacements "in vivo".

25. A scaffold as claimed in claim 24 in which fibres are oriented.

26. A scaffold as claimed in claim 24 in which fibres of different composition are layered.

27. Fibres as claimed in claim 19 aligned in parallel on a permeable flat surface (i.e. a semipermeable film) and seeded with cells.

28. Fibres as claimed in claim 19 as cell culture substrates for use in bioreactors also for freezing and thawing cells.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

CLAIMS

1. A method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

2. A method as claimed in claim 1 wherein the fibres have a length of 5mm-500mm.

3. A method as claimed in claim 1 wherein the fibres have a diameter of 5μm-1000μm.

4. A method as claimed in claim 1 wherein the depth of said channels is at least 1μm but not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre.

5. A method as claimed in [any one of claims 1 to 4] claim 1 wherein the width of the channel is no greater than half the radius of the fibre.

6. A method as claimed in [any one of claims 1 to 5] claim 1 wherein the channel formation extends longitudinally along the fibre.

7. A method as claimed in [any one of claims 1 to 5] claim 1 wherein the channel formations extend transversely to the longitudinal axis of the fibre.

8. A method as claimed in [any one of claims 1 to 5] claim 1 wherein channel is of U- "square-U", "rectangular-U" or V-shaped cross-section.

9. A method as claimed in claim 6 wherein, in transverse cross-section, the fibres comprise a plurality of lobes and said channel formation is defined between lobes of the fibre.

10. A method as claimed in [any one of claims 1 to 9] claim 1 wherein the cells locate on the open-mouths of the channel.

11. A method as claimed in claim 10 wherein said channel provides for guided growth of the cell along the channel.

12. A method as claimed in [any one of claims 1 to 9] claim 1 wherein the channels are dimensioned such that cells locate wholly within the channel.

13. A method as claimed in [any one of claims 1 to 9] claim 1 wherein the channels are dimensioned such that cells locate partly within the channel and partly above the profile of the fibre.

14. A method as claimed in [any one of claims 1 to 13] claim 1 wherein the fibres are in the form of a scaffold.

15. A method as claimed is claim 14 wherein the fibres are orientated.

16. A method as claimed in claim 15 wherein the fibres of different composition are layered.

17. A method as claimed in [any one of claims 1 to 13] claim 1 wherein the fibres are aligned as parallel on a permeable flat surface.

18. . A method as claimed in [any one of claims 1 to 17] claim 1 wherein said cells are selected from chondrocytes, cardiomyocytes, osteoblasts, myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchymal origin.

19. A fibre with an open-topped channel formation the depth of the channel being no more than 2/3 the diameter of the fibre but at least the width of an unspread cell (normally 10-20μ) and width no greater than ½ the radius.

20. A fibre as claimed in claim 19 that allows for cell adhesion and guided migration and growth.

21. A fibre with an open-topped channel formation in the form of a trough where the trough is at least 20 microns wide and 20 microns deep.

22. A fibre as claimed in claim 21 wherein the trough extends along the length of the fibre.

23. A fibre as claimed in [any one of claims 19 to 23] claim 19 in which various levels and gradients types of growth factor have been entrapped allowing diffusion to the surface to control growth.

24. A microfibrous cell scaffold composition comprising a fibre as claimed in any one of claims 19 to 23 for growing cells to produce functional tissue replacements "in vivo".

25. A scaffold as claimed in claim 24 in which fibres are oriented.

26. A scaffold as claimed in claim 24 in which fibres of different composition are layered.

27. Fibres as claimed in [any one of claims 19 to 24] claim 19 aligned in parallel on a permeable flat surface (i.e. a semipermeable film) and seeded with cells.

28. Fibres as claimed in [any one of claims 19 to 24] claim 19 as cell culture substrates for use in bioreactors also for freezing and thawing cells.

REMARKS

Consideration and allowance of the above-identified patent application is requested.

Respectfully submitted,

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PBA/NE/D088328PUS:TQH:135453

09/889717
JC18 Rec'd PCT/PTO 19 JUL 2001
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WO 00/47716 PCT/GB00/00144

FIBRES

The present invention relates to fibres and more particularly, but not exclusively, to fibres and materials comprised of such fibres for use in growing or culturing cells, e.g. for the purposes of tissue repair, in-vitro cell cultures and/or organ culture.

Medical devices that are implanted or used on broken skin in wound healing optimally require predictability in their interaction with surrounding tissues and blood. In some cases devices may act as templates for cell in-growth, in some cases they may have already been seeded with cells. Some devices require strong tissue in-growth for fixation, others may function best with minimal interaction. Understanding the biomaterial surface and the cell surface is clearly important in predicting what protein adsorption may occur and how cells may react in terms of adhesion, locomotion or active/passive response/transformation. The key determinants for the biomaterial surface will be surface chemistry in terms of hydrophobic/hydrophilic balance, surface charge, size and direction and counterion, physical size (macroscopic or microscopic), shape (geometry) and surface roughness/smoothness and mechanical properties (elasticity).

Substrates for tissue augmentation or to act as carriers for cultured cell transfer in wound therapy are usually collagen based. In this situation the collagen substrate usually has to be specific to the type of cell growth required and the phenotype and status (secretory, replicatory) grown on the substrate may not turn out to be as required.

It is the specific and non-specific physicochemical forces that will determine gene expression of the cell and phenotype the cell expresses will determine its status capability. It is known for example that cells grown on certain substrates will be inhibited from undergoing terminal differentiation, this has been shown for keratinocytes by Adams & Watt. So the attraction of using non-collagen surfaces for artificial cell substrates is that the cells may retain a migratory and genotypic

capability. The attachment to the cell whatever the substrate, collagen, vitronectin, thrombospondin will be through integrin cell-surface receptor.

Cells seeded onto the surface of a three dimensional structure may only grow in a two dimensional fashion or vice versa depending on the substrate. In some situations, for example nerve regeneration, it is desirable for there also to be not only predictability of attachment and growth but also direction. In the case of nerve regeneration this would mean that an axon could be directed to grow back down its original sheath. Similarly it may be desirable that a cell grow not only in a particular direction, but that a group of cells may grow in the same direction. In the case of voluntary muscle, the muscle belly is made up of muscle cells orientated with their long axes in parallel. Organs such as blood vessels rely on layers of muscle fibres with different layers orientated in a direction at 90° to the previous layer.

Historically work on guiding cells has shown that cells can be directed to migrate along the direction of surface deformations (scratches). Carter et alia (Hapto laxis), can be orientated by fluid shear (Eskin et alia, Ives et alia) by axial strain (Ives et alia). Others have shown that orientation of the Fn molecule can direct cell growth.

US patent 5,610,148 (Robert Brown) entitled "Macroscopically Orientated Cell Adhesion Protein" describes the production of a fibre comprised of fibrils of the cell adhesion protein fibronectin (Fn) that has been denatured and the polymer chains then aligned by unidirectional shear allowing aggregation and precipitation. Cells seeded onto these fibres demonstrate directional cell growth as a result of the longitudinal orientation of the cell adhesion-binding site.

According to a first aspect of the present invention there is provided a method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

The fibres may be used in the form a range of structures for use in providing cell growth, as described more fully below.

In accordance with the first aspect of the invention, therefore, culture of eukaryotic cells is effected on fibres (to the material of which the cells are able to attach) with an open-topped channel formation. Such a formation can be used to provide various advantageous effects for cell culture. Thus, for example, and as described more fully below, the dimensions of the cells may be so related to those of the channel formations that cells locate wholly within the channels and become attached therein to the material of the fibres. Such channel formations are also referred to herein as "troughs". In this case the cells are "protected" from the environment of the bulk phase in which the fibres are provided. Furthermore, the surface of the channel may be treated to provide particular advantageous effects for cell growth. The direction of cell growth may be along the length of the channel. Alternatively, the dimensions of the cells may be so related to those of the channel formations that cells "locate" on the mouth of the channel. Such channel formations are also referred to herein as "grooves". In this case, growth of the cell may be "guided" by the channel.

Routine "in vitro" cell culture has traditionally been carried out on non-porous, flat, 2 dimensional rigid substrates of low surface area to volume ratio. Fibres provide an opportunity to grow cells on high surface area to volume and to improve handling. Conditions of culture and by controlling surface chemistry and geometry of the fibre cell interactions can be predicted.

The cells may, for example, be selected from chondrocytes, cardiomyocytes, osteoblasts, myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchmal origin.

The fibres may be, but are not necessarily, of circular cross-section. The channel may extend generally linearly parallel to the axis of the fibre but other channel configurations which extend along the fibre length are also possible, e.g. helical. The

channel may extend along the full length of the fibre or terminate short of one or both ends thereof. The fibres may have two or more channels extending, for example, generally in parallel to each other. Alternatively the channel may extend transversely to the axis of the fibre.

The fibres may have a length of 1mm to 1000mm (more preferably 5mm-500mm) and preferably a diameter of 10μm-100μm).

It is preferred that the channel has a depth which is not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre but not less than the width of an unspread cell (generally considered to 10μm -20μm. The width of the channel is preferably no greater than half the radius. Such fibres provide a further aspect of the invention.

In certain embodiments of the fibre, the depth of the channel may be less than the width thereof. In other embodiments the channel depth may be greater than or equal to the width of the channel.

Preferred embodiments of fibre in accordance with the invention have a channel whereof the width and depth are each at least 20μm. Such fibres provide a further aspect of the invention.

The channel in the fibres may be of any desired configuration, e.g. U, "rectangular" or "square"-U, or V-shaped. The fibre may, in cross-section, comprise a plurality (e.g. 3) of lobes (preferably symmetrically disposed) and the channel formations are defined between adjacent lobes.

Examples of preferred dimension for a 30μm fibre are shown in the following tables:

Fibre + Groove

Width/Depth	Ideal range of 30μm fibre	Maximum groove size in relation to the fibre dimension	Minimum groove size in relation to cell dimension
Width	5-20μ	1/20 fibre circumference	1/20 cell circumference
Depth	3-10μ	1/10 fibre diameter	N/A

Fibre + Trough

Ideal range of 30μm fibre	Ideal range of 30μm fibre	Maximum trough size in relation to fibre dimension	Minimum trough size in relation to cell dimension
Profile	V.U		
Width	Normally 5-30μ	Fibre diameter <	1 x cell diameter
Depth	Normally 5-30μ	Radius ≤	1 x cell diameter

The above preferred dimensions may be readily adapted for fibre widths other than 30μm.

The fibre preferably comprises a biodegradable biopolymer or a combination of such polymer, examples of which include alginic acid salts (e.g. calcium alginate), carboxymethylcellulose, Methoxypectin, chitosan, chitosan derivatives (e.g. chitosan glutammate), and hyaluronic acid.

The channel of the fibre may have a coating of protein.

Fibres in accordance with the invention may be produced by spinning a solution of the material of which the fibre is to be formed through an orifice into a coagulation bath. The orifice may be configured such that the fibre solidifies into a

form incorporating a channel. Alternatively the orifice may be associated with an engraving device so that the channel is "cut" into the fibre as it is formed. In either case, the coagulation bath may incorporate a material to be adsorbed/absorbed into or onto the fibre surface. Alternative procedures for fibre formation include electrostatic spinning, solvent evaporation, and melt extrusion.

The channel formation ("groove" or "trough") may be formed by the following steps:

(1) The groove or trough is generated by extrusion of the solution through an orifice or a spinneret with a multitude of holes. The orifices may be circular with one or more grooves/troughs or a combination thereof. The grooves have preferably dimension of cell (width 5-10μm) or multiple thereof.

(2) The groove or trough may be produced by engraving the fibre with a sharp blade or engraver. Alternatively the incision can be made by passing the fibre through a rough surface. The roughness is given to the surface by micro-prominences (peaks convexities) having the shape of the groove/trough. They are positioned as the appropriate distance to engrave various fibres. The incision may be made at different stages of the production process of the fibres: after the filament precipitation, after stretching, after the washing, after the drying. It is advisable to groove the fibre after the filament has precipitated out in a short coagulation bath. It will subsequently re-coagulate in its engraved structure in another bath.

(3) The groove/trough can be obtained by etching.

The fibres may be formed into a structure, e.g. random matrices (e.g. non-woven felts and fleeces), orientated matrices (fibres having some relative alignment), knitted structure (e.g. knitted cloths), braided structures (e.g. braided thread), bundled structures, and carded slivers.

In preferred embodiments, microfibrous scaffolds are used that are composed of fibres designed to support cell attachment and directed cell motility and cell growth. The advantage of these fibres is they can be tailored to drive specific cell and tissue performance features by varying the level of surface charge, counterion presence or absence of growth factor, as well as their geometry, fibre orientation and axial strain; various fibres of the embodiments can be envisaged matrices, bundles, tubes, etc. The advantage of matrices made from these fibres is that the fibres can be random or have varying levels of orientation, layers of different types of fibre designed to suit different cell types can also be used. The fibres may also be mixed biodegradable and stable and vary in density and be shaped to form a template such that some mechanical, functional loading could be predicted that as degradation occurred would be counter balanced by appropriate tissue in growth. The advantages of these structures over collagen based substrates is that the seed cells once adhered will secrete and lay down there own extra-cellular matrix. Embodiments of the fibres with cell tracks or cell troughs that ensure that cells positioned at one end of the fibre will deliver to a point along the same fibre may be used in nerve regeneration where the damaged axon needs to grow back to its previous terminus. Besides non-woven matrices, embodiments may be knitted or woven structures that produce sheets or tubes useful in tissue repair or replacement, e.g. dermal substitute, vascular graft. Embodiments utilising the orientational/directional nature of the cell growth on these fibres and fibre scaffolds may be in vitro growth of muscle where muscle cell growth may be unidirectional along parallel fibre.

Following the principles outlined above a range of devices may be constructed from the fibres that are designed to allow cell attachment and growth (although it may be cell type specific) but is unique in that the fibre structure will cause the cell to grow down the fibre and thus be delivered to any point along, or at the other end of the fibre, for which we use the term "guide wire cell growth". The fibre structure that is unique in being able to predict this cell response is one where the bulk fibre maybe made of calcium alginate or other materials as described for different embodiments that has been formed with a groove along its length. The width of the fibre groove would normally be about 1/20 of the unspread circumference of the cell, the size of

the groove would normally be the diameter of an unstuck cell depending on the cell type but large enough to allow the cell to adhere and spread whilst retained within the groove. It is possible that the cell profile may stay entirely within the groove and not be above the circumferential profile of the fibre. In the past various methods have been proposed or actually utilised hollow fibre structures to grow cells and overcome some of the problems of growth on the surface of the fibre alone. The current grooved fibre structure proposed gives the benefits of both a fibre and hollow fibre in that the cells are in direct contact with the media whilst on a fixed and potentially permeable substrate but the level of surface fluid shear or mechanical shear to which the cell is exposed. If cells are placed on the inside of a hollow fibre allowing the cell to feed only by diffusion through the fibre wall there may be problems because of molecular weight cut off and tendency for the lumen of the main fibre to get blocked by cell growth reducing diffusion capability. In this circumstance the indent may be deep enough to be described as a trough. Depending on the size of the groove relative to the cell, the cell will tend to move along it.

The advantages to using an adhesion substrate other than collagen is that the cells once adhered and growing will lay down their own basement membrane and extra cellular matrix proteins, this eliminates the need for collagen specificity and the problems therein. Using synthetic substrates of the right chemistry it is also possible for the status of the cell and its phenotypic versus geotypic expression to be controlled.

By controlling the chemistry of the counterion of the fibre various cell types may preferentially adsorb or be encouraged to move faster or spread/adhere more or tightly to the fibre. Using this fibre structure cells can be guided down into three-dimensional matrices or can be encouraged to arrive at a specific location. Cells of different types can be seeded to grow into different parts of the structure. Cells are thus oriented on the fibre but by orienting the fibre itself all the cells in a structure maybe oriented. Chemotactic/migratory gradients may also be set up along the length of the fibre in order to speed up response.

It has also been shown that cells that are subjected to axial stress will align across the direction of stress so in an embodiment where cells are grown on an elastic fibre and have been allowed to grow into and between fibres to a point where a three cell structure has been formed by applying axial stress to the fibres the cells will be encouraged to align across the fibres to minimise the exposure. Using this technique for instance a structure seeded with muscle fibres could be organised so that the muscle cell contraction will produce a directional contraction and shortening. In some circumstances it may be appropriate to seed cells onto the fibres in a proteinaceous media in another single or multi protein solution depending upon the activity required. Similarly the fibre may be made of a biodegradable material e.g. biopolymers such as alginate or chitosan gelatin guar gum, etc. or synthetic polymers such as polylactic, polyglycolic or a non-degradable such as polyurethane, etc.

Uses of the cell growth procedures in accordance with the invention are set out below.

Wound Therapy

In an embodiment for wound therapy a bundle of fibres with troughs may be seeded with keratinocytes allowed to grow whilst suspended in culture media and then fibres laid singly in parallel across the wound. Cells in the trough are protected from the shear and damage of culture and handling and readily lay down a new basement membrane when placed on the wound.

Bioreactors

In an embodiment for bioreactors, the use of fibres with troughs provides a structure that would provide the ideal mammalian cell bioreactor. Cells can remain below the circumferential profile of the fibre and thus still be exposed to the culture media for diffusion of nutrients but not to the surface shear. For some cell types and with the appropriate substrate it may be possible to use axial stress to stimulate, align disrupt or detach cells depending on the amount of stretch.

Nerve Guidance

In an embodiment for nerve guidance the fibres would be structured with a trough the size appropriate to the axon and bundles of fibres oriented in parallel with the end of the fibre being in the same position at both proximal and distal positions. Fixation of bundle so that the ends were in apposition to the two ends of the cut nerve would allow directional and positional regeneration of the nerve.

Vascular Grafts

Grooved fibres would be formed into a tube by either winding onto a mandrel or knitting. By combining trough fibres with groove fibres cell alignment can be controlled by both fibres direction and axial stress so the desired layers of orientation could be obtained.

The invention is illustrated by the following non-limiting Examples and Figures of the accompanying drawings which show the results of the Examples.

For the Examples, the following procedures were used.

Cell Culture

L929 mouse fibroblast cells for use in an experiment were grown to confluence and then released from the tissue culture dishes by washing with Hepes Saline, followed by treatment with 0.25% trypsin solution. The resulting supernatant in centrifuged and the pellet of cells re-suspended in Dulbecco's modified Eagle's Medium [containing 10% Foetal calf serum, 5% Penicillin/Streptomyocin, 1% ITS (Insulin transferrin selenite)]. If being sub-cultured, then the cells were plated out on tissue culture plates at a 1:5 dilution.

15mg of each fibre type to be tested were weighed out and placed in each well of a 12 well tissue culture dish. In all experiments the fibres were washed in serum

containing media for a period of 24 hours. The experimental controls are cells plated on tissue culture plastic. Cells used for fibre testing were plated out at a density of 80,000 cells per well. All experiments were terminated up to a 72 hour timepoint.

Fixation and Staining

The cells and fibres were washed twice in Phosphate Buffered Saline (PBS) and then fixed using formalin solution (10% neutral buffered) for 10 minutes. The fixative is removed and the cells and fibres washed twice more in PBS. The cells were then stained with Geimsa for 10 minutes, followed by 3, five minute washes in PBS. The cells are then viewed using a Nikon Diaphot microscope and images captured using a JVC DV1 digital camcorder. The images were then downloaded to an Apple Macintosh Power PC Performa 6400/200 and analysis performed using the public domain program NIH image. For the scanning electron microscopy, fixed samples were dehydrated in 100% ethanol for a period of 2 hours. The samples are then sputter coated using a Denton Vacuum desk 1. The samples are mounted on a stub and viewed using a Hitachi S-510 scanning electron microscope. Images are captured using the JVC camera and analysed on the Macintosh computer using NIH image.

Example 1

Crabyon is a commercially available fibre (Omikenshi Co. Ltd., Osaka Japan) that is produced by co-spinning the polysaccharides chitin and chitosan with cellulose (rayon) by the well known viscose process. A number of grades of Crabyon are available with varying ratios of chitin and chitosan to rayon and with various fibre sizes. One such grade having a low (<50%) chitin/ chitosan content, a mean fibre length of 38mm and a diameter of 8-15μ was observed under a scanning electron microscope and was found to have continuous grooves of 1-2μ width along the length of the fibres (see figure 1a). The cell adhesion properties of Crabyon fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the Crabyon fibres was observed after 1 to 2 hours and many cells were elongated and aligned along the grooved fibres. After 72 hours much higher levels of cell attachment and elongation were observed (see figure 2b).

A measurement of the number of aligned and rounded cells adhering to the Crabyon fibres was made by taking an average of the number of adhered cells from 5 fields of view (the results are presented in figure 2c).

Example 2 (Comparative)

Cotton wool (cellulose) fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 10-20μ and to be smooth, flattened with few outstanding surface topographical features (see figure 2). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the fibres after 2hours was found to be very sparse, even after 48hours only a low degree of cell adhesion was observed with no evidence of cell elongation, alignment and spreading apparent.

Example 3

Chitosan, having a degree of de-acetylation >70% (available from Nigerian Fisheries), was spun as fibres from a solution of 3% w/w in 2% aqueous glacial acetic acid. The solution was ejected from a spineret having 20,000 holes each of 150μ diameter, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 10-20μ and to be smooth, cylindrical with few outstanding surface topographical features (see figure 3a). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the fibres after 2hours was found to be significant and spreading was evident but no elongation or alignment of cells was observed. After 48 hours the number of adherent cells had increased greatly but no evidence of elongation or alignment was apparent (see photograph taken from an optical microscope, figure 3b).

Example 4

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 3) from a 1ml insulin syringe through a needle of 35μ outside diameter into a coagulation bath of sodium hydroxide (5%w/w) and the resulting

fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 40-100μ and to be smooth, cylindrical with few outstanding surface topographical features (see figure 4). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Similar results to that cited in example 3 were obtained with a number of cells found to adhere to the fibres but no evidence for cell elongation and alignment was apparent.

Example 5

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 3) from a 50ml syringe through a needle of 500μ outside diameter that had been bent at the nozzle tip (with the intention of forming a groove in the fibre) into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope (figure 5a), the fibres were found to have a diameter of 200-300μ and to have a central channel of 100-150μ in width. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cells were found to attach to the fibre within the channel (see figure 5b)

Example 6

Chitosan fibres were made in the laboratory on a small scale spinning rig by ejecting a solution of chitosan (as specified in example 3) from a 50ml syringe through a spinneret of 1.2cm diameter having 20 holes each of 70μ diameter, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 20-50μ and to have grooves across the fibre of 5-10μ in width. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above and cells were found to adhere within the grooves of the fibre (figure 6).

Example 7

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 4) from a 20ml syringe through a spinneret of 1.2cm diameter having 756 trilobal shaped holes, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The dimensions of the spinneret were 3 symmetrical legs each of 70μ in length and 25μ in width (supplied by Enka Technica, Coventry, UK). The fibres were observed under the scanning electron microscope (figure 7a), and were found to have channels with a geometry resembling that of the trilobal spinneret. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. After two hours a high degree of cell attachment was observed with some cells spreading, although there was no evidence of cell elongation or orientation. After 72 hours there was very high cell attachment to the fibres (figure 7b).

Example 8

The procedure outlined in example 8 was repeated but the fibres were maintained in a hydrated state (in de-ionised water) before the cell adhesion properties were assessed. After two hours a high degree of cell attachment was observed with a great deal of cell spreading evident and some cells were observed to be elongated and orientated along the channel of the fibre. After 72 hours there was high cell attachment to the fibres. A comparison of the number of cells adhering to the fibres described in examples 1, 2, 7 and 8 was carried out after 2 hours and 72 hours, with the fibres of chitosan having a trilobal channeled structure proving more adherent (the results are portrayed in figure 8).

Figure Legends For Examples

Figure 1a
Rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi). The fibres 8 to 15 microns in diameter and are cylindrical. The topography on the surface is longitudinal grooves of width 1 to 2 microns.

Figure 2b
Rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi). The fibres 8 to 15 microns in diameter and are cylindrical. The topography on the surface is longitudinal grooves of width 1 to 2 microns. L929 fibroblasts can be seen to be attached to the fibres, one cells is oriented to the groove long axis and the other cell remains spherical, but well adhered.

Figure 1c
Graph depicting the morphology of cells attached to rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi) after a 72 hour timepoint. Cells show a preference to align to the longitudinal groove on the fibres surface. Data represents the mean of 5 fields of view. Error bars=standard deviation.

Figure 2
Cotton Wool composed of cellulose fibres (ex Asda Superstores). The fibres are flat and have a smooth topography. The diameter of the fibres is between 10 and 20 microns.

Figure 3a
Chitosan fibres (MF2, ex AMS) with a level of deaceytlation greater than 70% from routine commercial production. The fibres are cylindrical and have a smooth topography. The diameter of the fibres is between 10 and 20 microns.

Figure 3b
The adherence of L929 fibroblasts on chitosan fibres (MF2, ex AMS) with a diameter between 10 and 20 microns. The cells are well adhered and spread, but exhibit no alignment to the longitudinal axis of the fibre.

Figure 4
Chitosan fibres with a higher deacetylation of 70%, from laboratory production. The fibre is cylindrical in shape and has few surface topographical features. The diameter of the fibres is between 50 and 100 microns. L929 fibroblasts can be seen attached to the surface of the fibre.

Figure 5a
Chitosan fibres with a higher deacetylation of 70%, from laboratory production. The topography of the fibre is a 100 micron wide channel running longitudinally along the long axis. The diameter of the fibres is between 200 and 300 microns.

Figure 5b
L929 fibroblasts adhesion to 100 micron laboratory production chitosan fibres. Cells can be seen in the channel of the fibre.

Figure 6
Chitosan fibres with a greater deacetylation of 70% from laboratory production. The fibres have grooves running across them. The diameter of the fibres was between 20 and 50 microns. Cells demonstrated a good adherence to the fibres and showed orientation to the grooves running across the fibres.

Figure 7a
Chitosan fibres with a greater deacetylation of 70% from laboratory production. The fibres can be observed to be trilobal in shape. The diameter of the fibres was between 150 and 200 microns and the width of the lobes was 70 microns.

Figure 7b
L929 fibroblast adhesion to trilobal chitosan fibres with a greater deacetylation of 70%. Cells were seen to be well attached to the surface of the fibres.

Figure 8
Graph showing the level of adherence of L929 fibroblasts to chitosan fibres of different geometrics. After 72 hours, the dehydrated trilobal chitosan fibres have the greatest level of cell attachement. Hydrated trilobal chitosan fibres also demonstrate a high level of adherence greater than that of dehydrated round chitosan and Craybon fibres.

CLAIMS

1. A method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

2. A method as claimed in claim 1 wherein the fibres have a length of 5mm-500mm.

3. A method as claimed in claim 1 wherein the fibres have a diameter of 5μm-1000μm.

4. A method as claimed in claim 1 wherein the depth of said channels is at least 1μm but not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre.

5. A method as claimed in any one of claims 1 to 4 wherein the width of the channel is no greater than half the radius of the fibre.

6. A method as claimed in any one of claims 1 to 5 wherein the channel formation extends longitudinally along the fibre.

7. A method as claimed in any one of claims 1 to 5 wherein the channel formations extend transversely to the longitudinal axis of the fibre.

8. A method as claimed in any one of claims 1 to 5 wherein channel is of U-"square-U", "rectangular-U" or V-shaped cross-section.

9. A method as claimed in claim 6 wherein, in transverse cross-section, the fibres comprise a plurality of lobes and said channel formation is defined between lobes of the fibre.

10. A method as claimed in any one of claims 1 to 9 wherein the cells locate on the open-mouths of the channel.

11. A method as claimed in claim 10 wherein said channel provides for guided growth of the cell along the channel.

12. A method as claimed in any one of claims 1 to 9 wherein the channels are dimensioned such that cells locate wholly within the channel.

13. A method as claimed in any one of claims 1 to 9 wherein the channels are dimensioned such that cells locate partly within the channel and partly above the profile of the fibre.

14. A method as claimed in any one of claims 1 to 13 wherein the fibres are in the form of a scaffold.

15. A method as claimed is claim 14 wherein the fibres are orientated.

16. A method as claimed in claim 15 wherein the fibres of different composition are layered.

17. A method as claimed in any one of claims 1 to 13 wherein the fibres are aligned as parallel on a permeable flat surface.

18. A method as claimed in any one of claims 1 to 17 wherein said cells are selected from chondrocytes, cardiomyocytes, osteoblasts. myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchymal origin.

19. A fibre with an open-topped channel formation the depth of the channel being no more than 2/3 the diameter of the fibre but at least the width of an unspread cell (normally 10-20μ) and width no greater than ½ the radius.

20. A fibre as claimed in claim 19 that allows for cell adhesion and guided migration and growth.

21. A fibre with an open-topped channel formation in the form of a trough where the trough is at least 20 microns wide and 20 microns deep.

22. A fibre as claimed in claim 21 wherein the trough extends along the length of the fibre.

23. A fibre as claimed in any one of claims 19 to 23 in which various levels and gradients types of growth factor have been entrapped allowing diffusion to the surface to control growth.

24. A microfibrous cell scaffold composition comprising a fibre as claimed in any one of claims 19 to 23 for growing cells to produce functional tissue replacements "in vivo".

25. A scaffold as claimed in claim 24 in which fibres are oriented.

26. A scaffold as claimed in claim 24 in which fibres of different composition are layered.

27. Fibres as claimed in any one of claims 19 to 24 aligned in parallel on a permeable flat surface (i.e a semipermeable film) and seeded with cells.

28. Fibres as claimed in any one of claims 19 to 24 as cell culture substrates for use in bioreactors also for freezing and thawing cells.

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FIG. 1a

FIG. 1b

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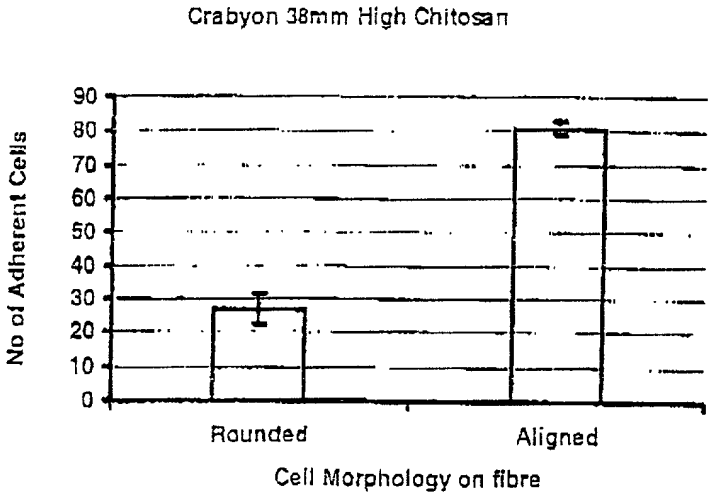

FIG. 1c

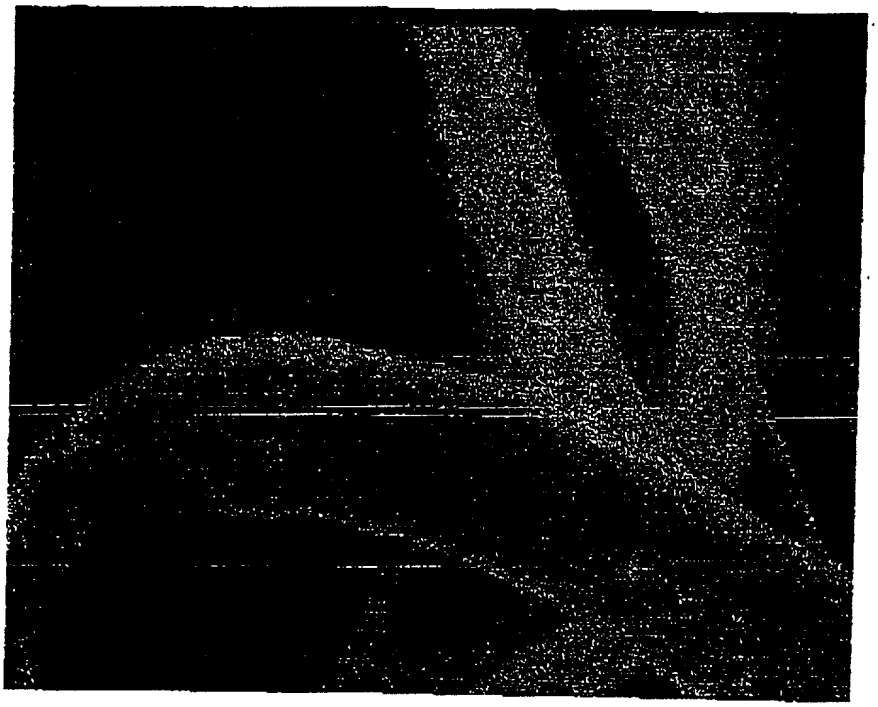

FIG. 2

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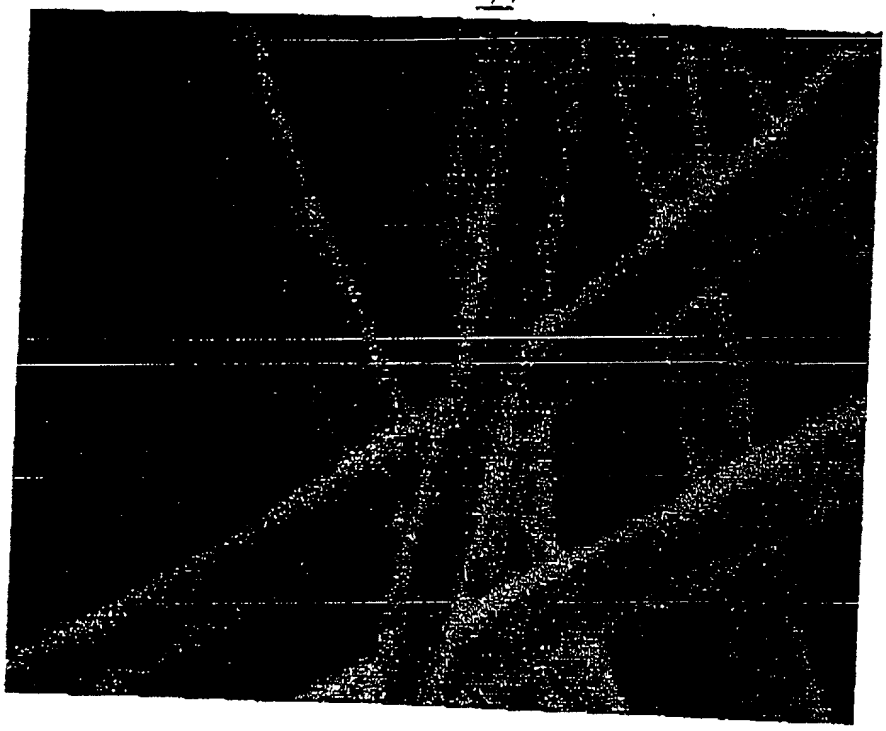

FIG. 3a

FIG. 3b

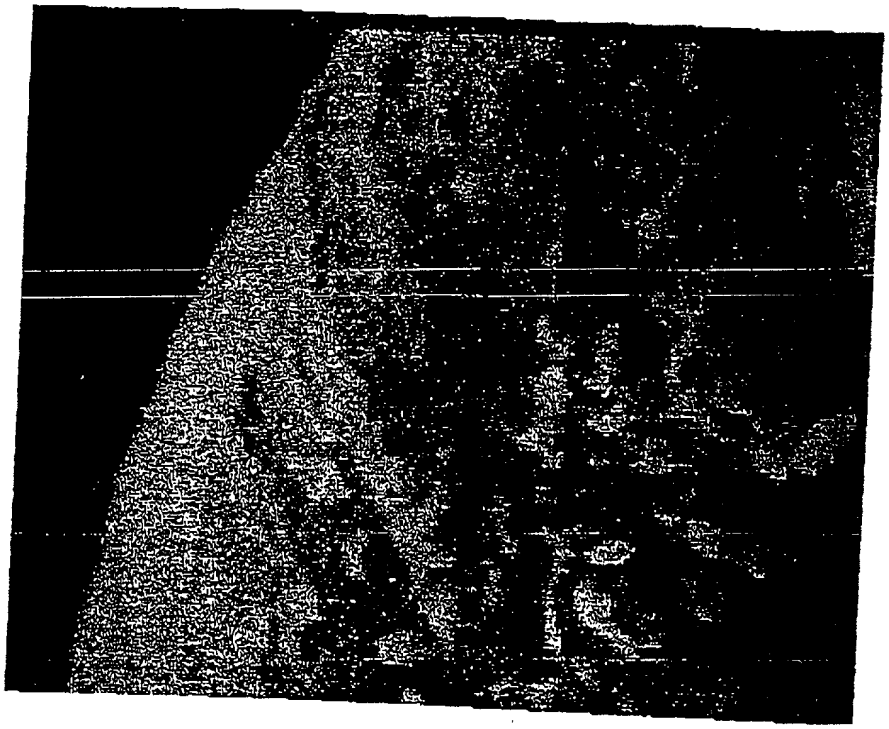

FIG. 4

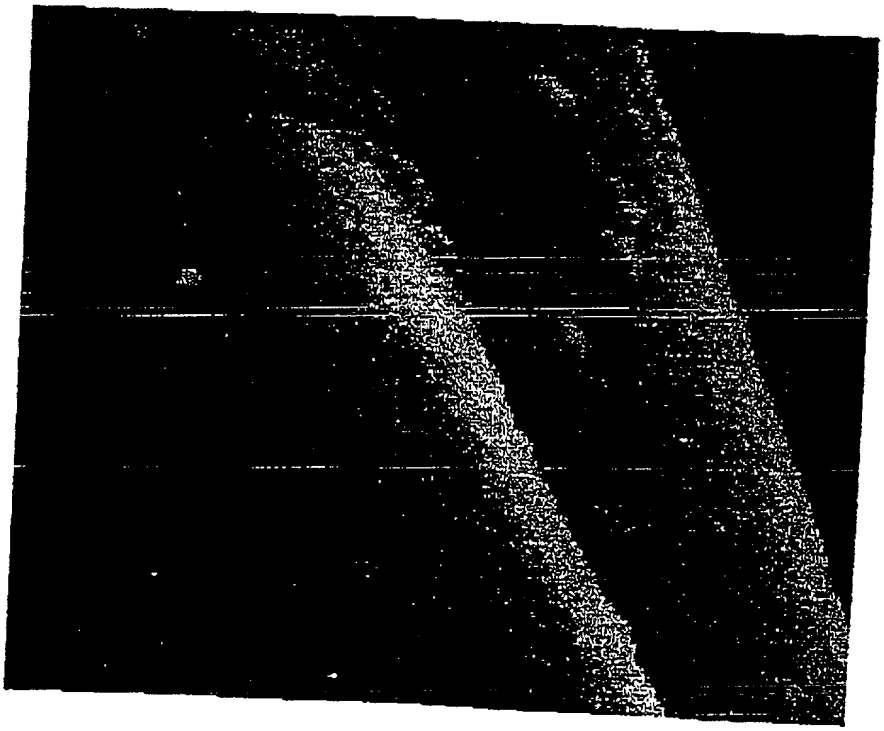

FIG. 5a

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FIG.5b

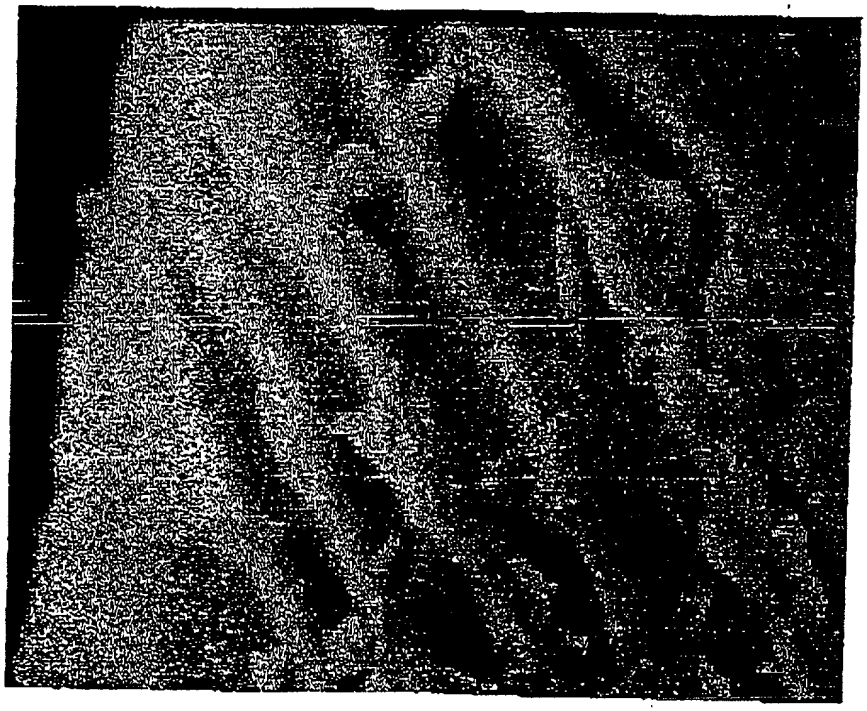

FIG.6

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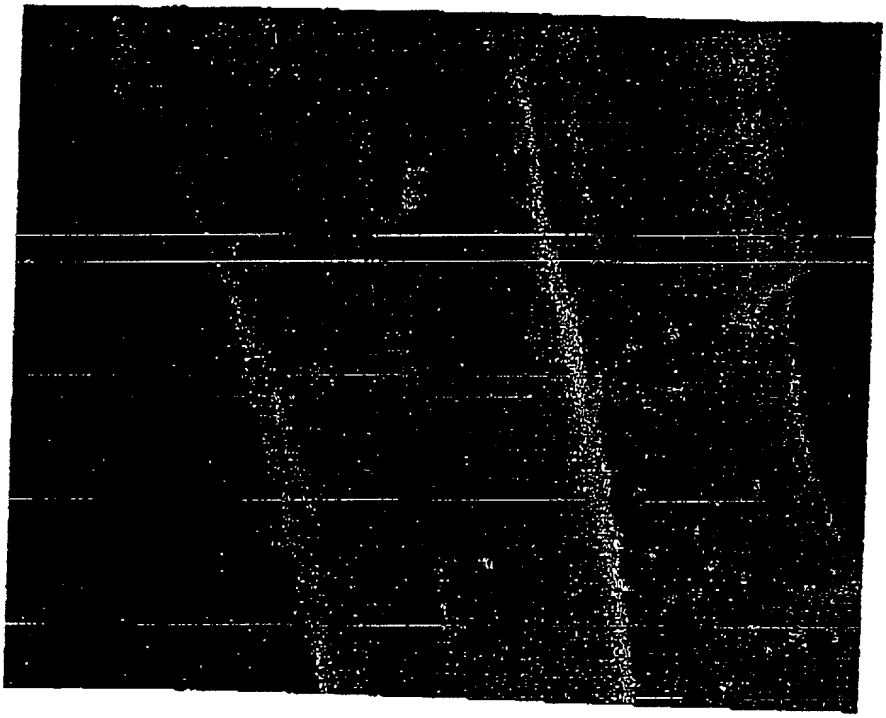

FIG.7a

FIG.7b

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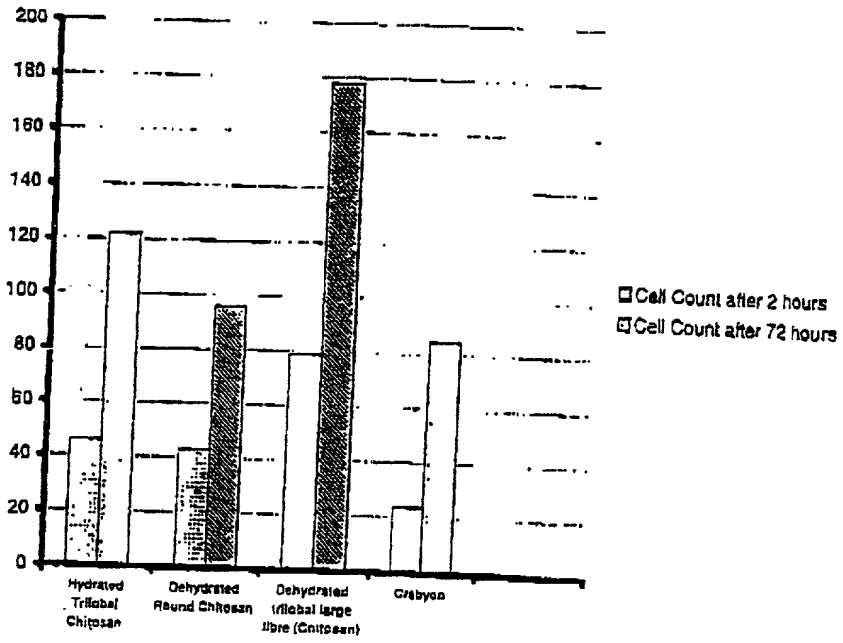

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FIG.8

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DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney Docket Number	7250-11
First Named Inventor	Douglas William Hamilton

JAN 28 2002 PATENT & TRADEMARK OFFICE

☐ Declaration submitted with Initial Filing

☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

COMPLETE IF KNOWN

Application No.	09/889,717
Filing Date	
Group Art Unit	
Examiner's Name	

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FIBRES

the specification of which
(check one)

☐ is attached hereto.

☒ Was filed on January 21, 2000 as United States Application No. or

PCT International Application No. PCT/GB00/00144

☐ And was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YY)	Priority Not Claimed	Certified Copy Attached? Yes	Certified Copy Attached? No
GB00/00144	PCT	01/21/00		☐	☒
GB 9901272.6	GB	01/21/99			
GB 9903561.0	GB	02/17/99		☐	☒

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

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Name	Registration Number	Name	Registration Number
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Inventor's Signature:		Date of Signature:	
Residence: (City, State, Country)			
Citizenship:			
Post Office Address:			

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WENMM SB/02C (3-97)

DECLARATION

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(Supplemental Sheet)

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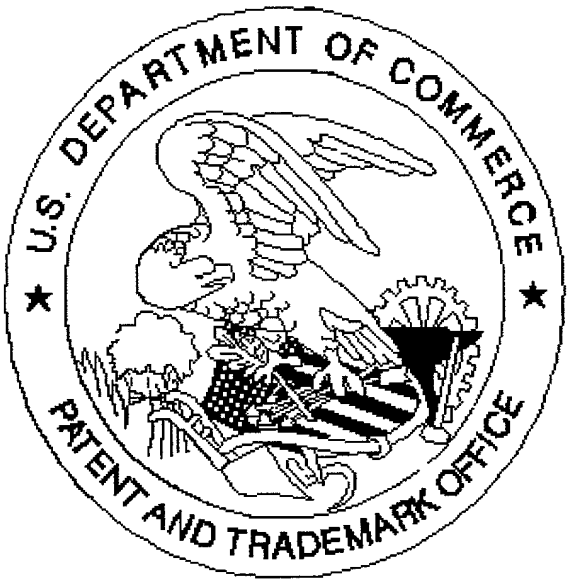

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